Claims

1. A method for determining an amino acid sequence motif or a peptidomimetic sequence motif containing an active site capable of being bound by an enzyme which catalyses covalent modification of a substrate molecule, comprising;

a) contacting the enzyme with a library consisting of a number of oriented degenerate library subsets of molecules, each subset comprising unmodified degenerate motif sequences each having n residues and each having a modifiable residue at a different fixed non-degenerate position, under conditions which allow for modification of molecules which are a substrate for the enzyme;

b) allowing the enzyme to modify modifiable residues in library subsets containing molecules having an active substrate site for the enzyme;

c) deconvoluting the oriented degenerate library subsets of the library, *in situ* without separating modified from unmodified molecules, so as to reveal the sequence of any motif which has been modified by covalent binding of the enzyme;

wherein each library subset is of formula (I)

$$(Xaa)_x\ Zaa\ (Xaa)_y \quad (I)\ [SEQ\ ID\ No.\ 1]$$

wherein

Zaa is a non-degenerate modifiable natural or unnatural amino acid residue or peptidomimetic;

Xaa is any natural or unnatural amino acid residue or peptidomimetic;

x and y are each independently 0 or an integer;

$(x + y) = (n-1)$; and

n = an integer from 3 to 8, preferably 5.

2. A method according to claim 1 which includes the further step of synthesising a substrate molecule containing a motif sequence revealed in step (c) or an analogue of said motif sequence.

3. A method according to claim 1 in which said revealed substrate molecule motif sequence, or an analogue thereof, is used to develop a selective inhibitor of said enzyme, which method includes the step of changing the modifiable residue to a derivative form of the residue which is not modifiable by the enzyme.

4. An enzyme substrate molecule produced according to the method of claim 2.

5. An enzyme inhibitor molecule produced according to the method of claim 3.

6. A pharmaceutical composition comprising as an active ingredient a substrate molecule according to claim 2.

7. A pharmaceutical composition comprising as an active ingredient an inhibitor molecule according to claim 3.

8. A method of treatment which includes administering to a patient an effective amount of a substrate molecule according to claim 2 or a composition according to claim 6.

9. A method of treatment which includes administering to a patient an effective amount of an inhibitor molecule according to claim 3 or a composition according to claim 7.

10. A method according to claim 1 wherein $x + y = (n-1) = 4$.

11. A method according to claim 1 or 10 wherein the step (c) of deconvolution is carried out according to the procedure for auto-deconvolution of combinatorial libraries described in WO 97/42216.

12. A method according to claim 1 wherein Formula 1 may optionally include at any place in the formula one or more invariant residue(s), said residue(s) being in the same relative position(s) in each subset of the library.

13. A method according to any of claims 1 to 3 and 8 to 12 wherein said enzyme catalyses covalent modification selected from the group consisting of phosphorylation, acylation; and dephosphorylation.

14. A method according to any of claims 1 to 3 and 8 to 13 wherein said enzyme is a protein kinase enzyme.

15. A method according to any of claims 1 to 3 and 8 to 14 wherein said modifiable residue Z is selected from the group consisting of tyrosine; serine; threonine; histidine; and aspartic acid.

16. A protein kinase inhibitor capable of inhibiting the catalytic transfer of the

γ-phosphate from ATP to an amino acid residue on a substrate molecule, said inhibitor having been produced by the method of any of claims 1 to 3 and 8 to 15.